Element Biosciences

Technical Note

Sequencing with Cloudbreak Freestyle™

Optimized chemistry maximizes compatibility, ease of use, and quality

Highlights

- Eliminates manual library conversion
- Compatible with > 95% of library prep kits
- Meets or exceeds Cloudbreak™ performance

Introduction

Avidity base chemistry (ABC) has revolutionized next-generation sequencing (NGS), making this invaluable genomics tool accessible to all labs by dramatically reducing costs and setting new standards for quality and flexibility.[1] Cloudbreak sequencing kits innovated on this core technology, introducing faster run times, greater accuracy, and onboard circularization of Elevate™ libraries.

Cloudbreak Freestyle kits continue the trajectory of rapid innovation, building on ABC and Cloudbreak to enable linear library loading and circularization on the AVITI™ System. From whole-genome sequencing (WGS) to single-cell studies, Cloudbreak Freestyle redefines ease of use and quality in benchtop sequencing.

Broader ecosystem compatibility

ABC provides two entry points to sequencing: the Element Elevate Library Prep Workflow for preparation of Element libraries and the Element Adept™ Library Compatibility Workflow for conversion of third-party libraries. The Adept Workflow follows a simple protocol to make any library ABC-compatible. For labs that continue to rely on the exceptionally broad compatibility of manual library conversion, Cloudbreak continues to support Adept libraries.

Cloudbreak Freestyle streamlines conversion, incorporating Adept components into the sequencing reagents so researchers can go from library prep with an Elevate kit or third-party kit directly to sequencing. The Cloudbreak Freestyle sequencing cartridge also replaces Adept or Elevate sequencing primers with universal sequencing primers, eliminating the need to swap primers before thawing reagents.

Cloudbreak Freestyle performance

AVITI FIT expanded the menu of Cloudbreak sequencing kits to open avenues for lower throughputs, strict budgets, and more applications. One for one, Cloudbreak Freestyle kit configurations match Cloudbreak kit configurations so that labs gain the same scalability and flexibility without compromising quality. Cloudbreak Freestyle delivers the same high-quality sequencing performance as Cloudbreak (Table 1) while greatly improving the sequencing workflow. Table 2 presents representative sequencing metrics across a selection of applications generated using Cloudbreak Freestyle.

Sequencing Kit	Read Count[a]	Run Time (hours)[b]	%Q30
AVITI 2x150 Sequencing Kit Cloudbreak High Output	1 billion	38	> 90%
AVITI 2x150 Sequencing Kit Cloudbreak Freestyle High Output	1 billion	38	> 90%

[a] Read counts are based on sequencing Element libraries. Actual read counts might differ based on lab-specific factors.
[b] Individually addressable lanes and other custom recipes can extend run times.

Table 1. Comparison of sequencing metrics

	10X Flex	RNA-Seq		WGS			WES	
	Cloudbreak Freestyle	Cloudbreak Freestyle	Cloudbreak Freestyle	Cloudbreak Freestyle	Cloudbreak Freestyle	Cloudbreak Freestyle	Cloudbreak Freestyle	Cloudbreak Freestyle
Library type	Linear third party	Linear Elevate	Linear third party	Linear PCR-free Elevate ~300 bp insert	Linear PCR-free third party ~300 bp insert	Linear PCR-free third party ~500 bp insert	Linear Elevate	Linear third party
Loading	7.5 pM with 0.1 pM PhiX	7.5 pM with 0.1 pM PhiX	8.5 pM with 0.1 pM PhiX	7.5 pM with 0.5 pM PhiX	7.5 pM with 0.5 pM PhiX	8.5 pM with 0.2 pM PhiX	7 pM with 0.1 pM PhiX	9 pM with 0.1 pM PhiX
Format	29 x 91	2 x 75	2 x 75	2 x 150	2 x 150	2 x 150	2 x 75	2 x 75
Density	1026.1 M	1061.8 M	1029.6 M	1099.1 M	1037.6 M	947.2 M	1089.9 M	1034.9 M
%Q30	98.6%	97.0%	94.3%	95.6%	94.8%	94.6%	96.4%	97.0%
Index assignment	98.6%	95.9%	94.7%	98.4%	96.6%	96.4%	96.9%	96.3%

Table 2. Cloudbreak Freestyle demonstrates consistent high-quality results across sequencing applications

Flexibility without compromising quality

Element Biosciences evaluated method-appropriate performance metrics across third-party human WGS, human whole-exome sequencing (WES), RNA sequencing (RNA-Seq), and single-cell sequencing libraries to demonstrate equivalence between Cloudbreak and Cloudbreak Freestyle.

Library prep methods and input are presented in Table 3. Following library prep, the Adept 1.1 protocol was used to convert libraries for Cloudbreak sequencing, while sequencing matched linear libraries using Cloudbreak Freestyle.

Libraries were diluted to the target loading concentration with a 0.1 pM–0.5 pM spike-in of PhiX Control Library and sequenced on the AVITI System.

Method	Library Type	Samples	Library Prep	Indexes
Human WGS, (400 bp and 500 bp insert)	Third party	HG001[a]	KAPA Hyperplus Kit	KAPA Unique Dual-Indexed Adapter Kit
Human WES	Third party	HG001	Twist Exome 2.0	Twist Universal Adapter System
RNA-Seq	Third party	UHRR and HBRR[b]	KAPA mRNA HyperPrep Kit	KAPA Unique Dual-Indexed Adapter Kit
Single-Cell Sequencing	Third party	BC1 PBMC[c], BC2 PBMC, BC3 BMMC[c], and BC4 Isolated T cells	10X Chromium Single Cell Expression Flex	

[a] Genome in a Bottle human reference sample
[b] Universal Human Reference RNA and Human Brain Reference RNA
[c] Peripheral blood mononuclear cells and bone marrow mononuclear cells

Table 3. Library prep information for each NGS application

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WGS

Cloudbreak and Cloudbreak Freestyle were each used to perform whole-genome sequencing (WGS) on third-party libraries using human reference genomic DNA.

PCR-free libraries were prepared from 1 µg of HG001 using the KAPA HyperPlus Kit. The samples were mechanically sheared to ~400 or 500 bp, end-repaired and ligated with KAPA unique dual index (UDI) adapters. Libraries sequenced with Cloudbreak chemistry were first converted using the Element Adept Library Compatibility Kit v1.1.

Libraries were sequenced on the AVITI System with a 2 x 150 read length. Bases2Fastq was used to generate FASTQ files.

Samples were downsampled to 35X and then aligned using Sentieon BWA. Benchmarking was accomplished using Google DeepVariant 1.6 and NIST v4.2.1 truth VCF/BED.

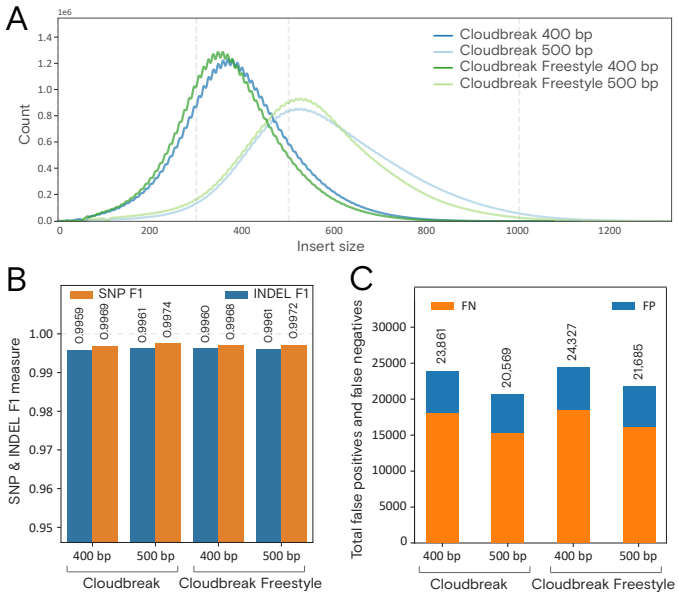

Figure 1. Comparing 400 bp and 500 bp insert length whole-genome sequencing metrics between Cloudbreak and Cloudbreak Freestyle. (A) Aligned insert length, (B) SNP and INDEL F1 scores, and (C) Total errors.

WES

Cloudbreak and Cloudbreak Freestyle were each used to perform human whole-exome sequencing (WES) on third-party libraries using human reference gDNA.

The Twist Library Preparation Enzymatic Fragmentation (EF) Kit 2.0 was used to prepare precapture libraries from input of 50 ng of HG001 gDNA. Eight replicates were simultaneously prepared with unique dual indexes (UDIs). Replicates were pooled equally and captured together using the Twist Exome 2.0 panel and Standard Hybridization v2 Protocol. Captured libraries sequenced with Cloudbreak were first converted using the Element Adept Library Compatibility Kit v1.1.

Libraries were sequenced on the AVITI System with a 2 x 75 read length. Bases2Fastq was used to generate FASTQ files.

FASTQs were downsampled to 40M reads and aligned using Sentieon BWA. Benchmarking was accomplished using Google DeepVariant 1.6 and NIST v4.2.1 truth VCF/BED.

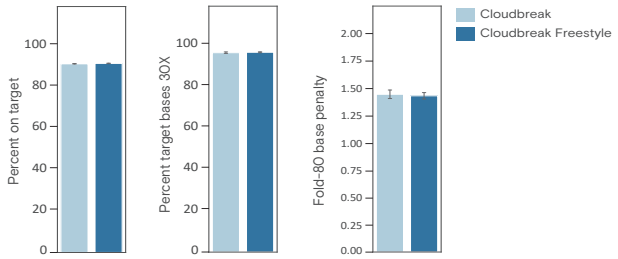

Figure 2. Exome secondary analysis metrics comparing Cloudbreak and Cloudbreak Freestyle sequencing.

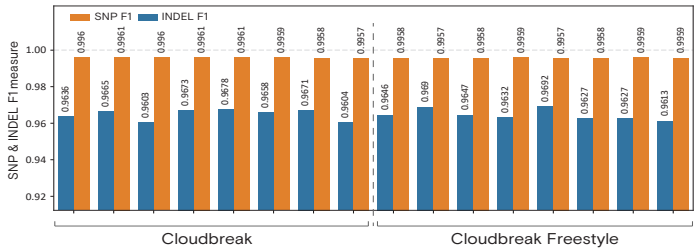

Figure 3. SNP and INDEL F1 scores for an 8-plex whole-exome library comparing Cloudbreak and Cloudbreak Freestyle sequencing.

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RNA-Seq

Cloudbreak and Cloudbreak Freestyle were used to perform RNA sequencing (RNASeq) on third-party libraries using human reference RNA samples. Libraries were prepared using the KAPA RNA HyperPrep Kit and 100 ng of Universal Human Reference RNA and Human Brain Reference RNA (Thermo Fisher). The samples were enzymatically sheared to ~300 bp, end-repaired, and ligated with KAPA unique dual index (UDI) adapters.

Libraries sequenced with Cloudbreak were first converted using the Element Adept Library Compatibility Kit v1.1. Libraries were sequenced using Cloudbreak Freestyle chemistry on the AVITI System with a 2 x 75 read length. Bases2Fastq was used to generate FASTQ files.

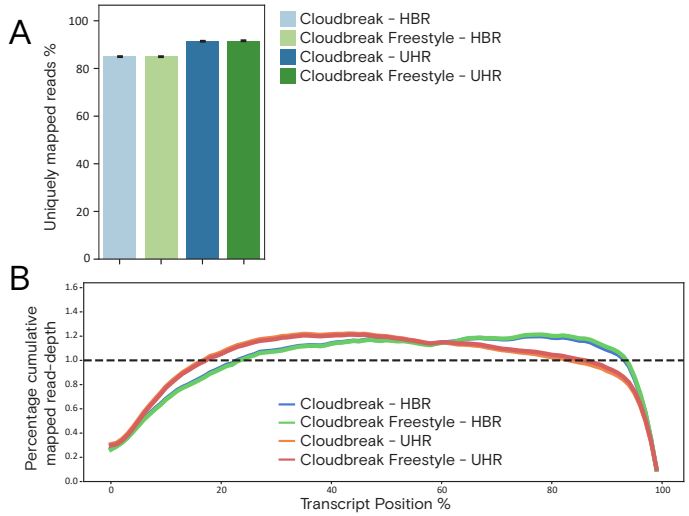

Figure 4. Comparing (A) Uniquely Mapped Read % and (B) 5' to 3' transcript coverage from RNA sequencing of UHR and HBR using Cloudbreak and Cloudbreak Freestyle.

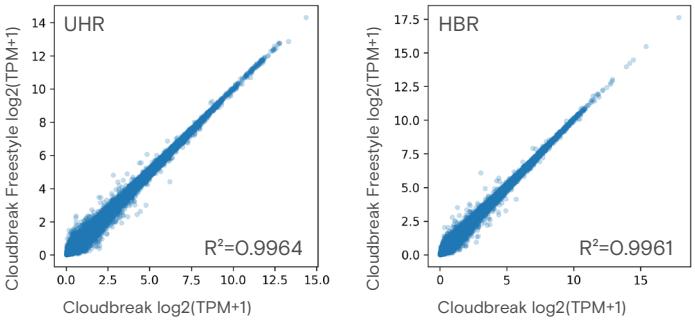

Figure 5. Transcript abundance correlations of UHR and HBR libraries sequenced using Cloudbreak and Cloudbreak Freestyle.

Single-cell sequencing

Cloudbreak and Cloudbreak Freestyle chemistry were used to perform single-cell gene expression sequencing, profiling fixed samples. The 10x Genomics Chromium Single Cell Gene Expression Flex Kit was used to prepare libraries from human peripheral blood mononuclear cells (PBMC), human T cells, and bone marrow mononuclear cells (BMMC).

Libraries were pooled to target ~10,000 reads per cell and sequenced with Cloudbreak and Cloudbreak Freestyle chemistry on the AVITI System using a 29 x 91 cycle run.

Libraries sequenced with Cloudbreak were first converted using the Element Adept Library Compatibility Kit v1.1. Bases2Fastq was used to generate FASTQ files. 10x Genomics prepared the samples and libraries, which Element sequenced and analyzed using Cell Ranger v.7.1.

10X Cellranger Count Metrics v7.1	Cloudbreak	Cloudbreak Freestyle
Estimated number of cells	43,831	43,912
Median genes per cell	2640	2692
Number of reads	920,995,188	1,006,385,282
Q30 barcodes	98.9%	98.4%
Q30 UMI	98.4%	97.9%
Q30 RNA read	98.9%	98.2%
Confidently mapped reads in cells	96.7%	96.7%

Table 4. AVITI performance using Cloudbreak and Cloudbreak Freestyle sequencing on a 40K cell sample comprised of human peripheral blood mononuclear cell (PBMC), human T cells, and bone marrow mononuclear cells (BMMC), analyzed with Cell Ranger v7.1.

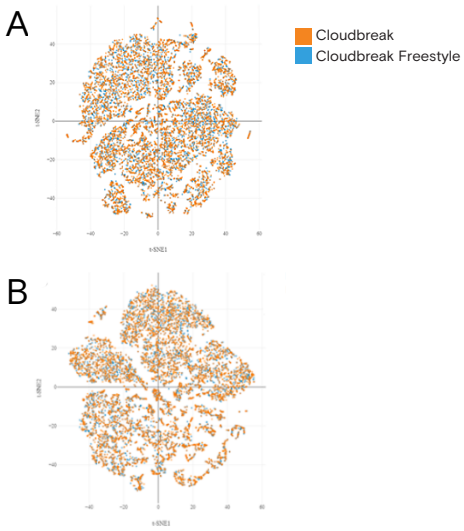

Figure 6. t-SNE plots for PBMC (A) and BMMC (B) showing strong overlap of cell populations measured with Cloudbreak and Cloudbreak Freestyle.

Summary

Cloudbreak introduced onboard circularization of Elevate libraries, replacing manual circularization with automatic circularization that occurs as part of the sequencing run without additional run time.

Cloudbreak Freestyle expands this capability, enabling onboard circularization of most libraries, including those prepared with third-party kits. Building on the core ABC technology, Cloudbreak Freestyle sequencing kits deliver high-quality performance while enhancing compatibility and usability.

Cloudbreak Freestyle continues fixed reagent pricing, low per-run cost, individually addressable lanes, and other precedents established with Cloudbreak. Upgrading to Cloudbreak Freestyle leverages these same advantages while adding enhancements to accelerate your science.

To learn more, visit elementbiosciences.com

References

1. Arslan, Sinan, Francisco J. Garcia, Minghao Guo, et al., "Sequencing by avidity enables high accuracy with low reagent consumption," *Nature Biotechnology* (May 2023): https://doi.org/10.1038/s41587-023-01750-7.

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